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Detection of SARS-CoV-2 in the air in Indian hospitals and houses of COVID-19 patients

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ABSTRACT

To understand the transmission characteristics of severe acute respiratory syndrome corona virus-2 (SARS-CoV-2) through air, samples from different locations occupied by coronavirus disease (COVID-19) patients were analyzed. Three sampling strategies were used to understand the presence of virus in the air in different environmental conditions. In the first strategy, which involved hospital settings, air samples were collected from several areas of hospitals like COVID-intensive-care units (ICUs), nurse-stations, COVID-wards, corridors, non-COVID-wards, personal protective equipment (PPE) doffing areas, COVID rooms, out-patient (OP) corridors, mortuary, COVID casualty areas, non-COVID ICUs and doctors' rooms. Out of the 80 air samples collected from 6 hospitals from two Indian cities- Hyderabad and Mohali, 30 samples showed the presence of SARS-CoV-2 nucleic acids. In the second sampling strategy, that involved indoor settings, one or more COVID-19 patients were asked to spend a short duration of time in a closed room. Out of 17 samples, 5 samples, including 4 samples collected after the departure of three symptomatic patients from the room, showed the presence of SARS-CoV-2 nucleic acids. In the third strategy, involving indoor settings, air samples were collected from rooms of houses of home-quarantined COVID-19 patients and it was observed that SARS-CoV-2 RNA could be detected in the air in the rooms occupied by COVID-19 patients but not in the other rooms of the houses. Taken together,

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we observed that the air around COVID-19 patients frequently showed the presence of SARS-CoV-2 RNA in both hospital and indoor residential settings and the positivity rate was higher when 2 or more COVID-19 patients occupied the room. In hospitals, SARS-CoV-2 RNA could be detected in ICUs as well as in non-ICUs, suggesting that the viral shedding happened irrespective of the severity of the infection. This study provides evidence for the viability of SARS-CoV-2 and its long-range transport through the air. Thus, airborne transmission could be a major mode of transmission for SARS-CoV-2 and appropriate precautions need to be followed to prevent the spread of infection through the air.

1. Introduction

The ~100 nm viral particle, SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2 has affected different aspects of human life in multiple ways (Bar-On, Flamholz et al., 2020). The effects on the patient are not only restricted to the dysfunction of the respiratory system but are seen to range from the patient being totally asymptomatic to respiratory or multiorgan failure in several cases (Baj, Karakuła-Juchnowicz et al., 2020). In spite of the multiple measures taken by countries globally, containing the virus has been challenging. Initially, contact and droplet-mediated transmission were considered as major modes of transmission for the SARS-CoV-2. Accordingly, to avoid contracting the disease, hand washing and social distancing were the main measures suggested along with wearing masks. The alarmingly increasing number of cases of COVID-19 globally raised the possibility of airborne transmission of SARS-CoV-2 (The Lancet Respiratory, 2020). The antecedent of SARS-CoV-2, SARS-CoV-1, which had an outbreak in 2002–2004, was reported to spread through air (Li, Huang et al., 2005; Yu, Wong et al., 2005) and through viral aerosols generated by patients (Booth, Kournikakis et al., 2005). Apart from SARS-CoV-1, other viruses - Norwalk like virus (Marks, Vipond et al., 2003), respiratory syncytial virus (Kulkarni, Smith et al., 2016), MERS (Middle East Respiratory Syndrome) coronavirus (Kim, Chang et al., 2016) and Influenza A/H591 virus between ferrets (Herfst, Schrauwen et al., 2012) were also reported to have the possibility of airborne transmission. Considering that these viruses, especially SARS-CoV-1 and MERS virus, which are closely related to SARS-CoV-2, were capable of getting transmitted through air, the possibility of airborne transmission of SARS-CoV-2 cannot be ruled out (Morawska & Cao, 2020; The Lancet Respiratory, 2020). It has been observed that SARS-CoV-2 is quite stable in aerosols (van Doremalen, Bushmaker et al., 2020) and that it is more stable in aerosols when compared to SARS-CoV-1 and MERS (Fears, Klimstra et al., 2020).

There are existing reports of surface and environment contamination with SARS-CoV-2 in hospital rooms of COVID-19 positive patients (Birgand, Peiffer-Smadja et al., 2020; Chia, Coleman et al., 2020; Liu, Ning et al., 2020; López, Romo Á et al., 2020). A study by Lednicky et al. (2020) has provided evidence for the presence of viable SARS-CoV-2 in the air samples collected from hospital room with COVID-19 patient even in the absence of any aerosol generating procedure (Lednicky, Lauzardo et al., 2020). Rami et al. (2020) also suggested that, in hospitals, droplets containing SARS-CoV-2, with strong directional airflow, can spread the virus farther than 2 m (Sommerstein, Fux et al., 2020). A study in Wuhan found that air samples collected using robot assisted air sampling from hospitals, were positive for SARS-CoV-2 and COVID-19 patients who had recovered and were ready for discharge, exhaled the virus in the air (Zhou, Yao et al., 2021). A systematic review of 73 studies published on presence of SARS-CoV-2 RNA in air suggested that the concentration of SARS-CoV-2 RNA was higher in indoor air as compared to outdoor air and in indoor, it was higher in hospital and healthcare settings as compared to that in community indoor settings (Dinoi, Feltracco et al., 2022). A pilot study on environmental sampling of SARS-CoV-2 in indoor residential settings involving two self-quarantined COVID-19 patients detected SARS-CoV-2 RNA in the air in the rooms (Nannu Shankar, Witanachchi et al., 2022). Another study involving non-healthcare setting, a university dormitory housing quarantined individuals, detected SARS-CoV-2 RNA in surface swabs collected from the HVAC filters and bathroom exhaust grilles (Pan, Hawks et al., 2022).

To get further insights on transmission characteristics of SARS-CoV-2 in air in hospital and indoor residential settings, and to assess the risk for healthcare workers and the caretakers, we analyzed air samples using three sampling strategies- i) air sample collection from different locations of hospitals, ii) air sample collection from closed rooms in which either one or more COVID positive individuals spent only short time, and iii) air sample collection from rooms of houses of home-quarantine COVID-19 positive individuals. We found that SARS-CoV-2 RNA is detected in the air in hospital and residential indoor settings housing COVID-19 patients. It is detected at different distances from the patient, over different time scales. The study provides evidence for the long-range transport of the SARS-CoV-2 through the air. Importantly, the virus collected from air could infect cells in culture, thus, hinting towards the possibility of airborne transmission for SARS-CoV-2.

2. Methods

Ethical approval

The study was approved by the Institutional Ethics Committee of CSIR- Centre for Cellular and Molecular Biology (IEC-83/2020). Informed consent from subjects was taken wherever required.

2.1. Hospital settings

Air samples were collected from three hospitals from Hyderabad, India - Hospital 1, Hospital 2 and Hospital 3, in between September 2020 to January 2021; and three other hospitals from Mohali, India –Hospital 4, Hospital 5 and Hospital 6 in between July 2020 to December 2020. Hospital 1 is a large tertiary care government hospital, being exclusively used for COVID care at the time of sample collection. Hospital 2 is a private trust hospital catering to very limited number of COVID patients while Hospital 3 is a tertiary care government set-up catering to a moderate number of COVID patients along with other medical services. Hospital 4, Hospital 5 and Hospital 6 are all tertiary care hospitals catering to moderate number of COVID and non-COVID patients. Air samples were collected from COVID-intensive-care units (ICUs) occupied by COVID-19 patients, nurse stations, COVID wards, corridors, non-COVID wards, PPE doffing areas, COVID rooms, OP corridors, mortuary, COVID casualty areas, non-COVID ICUs and doctors' rooms. The details of air sampling conditions are mentioned in Data in brief Tables 2 and 3.

2.2. Closed-room controlled experiments

Asymptomatic or mildly symptomatic COVID-19 positive individuals were asked to sit in a closed room during air sample collection procedure without wearing masks (Fig. 1). The mildly symptomatic individuals had mild cough and fever, but did not require hospitalization. Immediately after sample collection procedure was over, they were allowed to leave the room. Air samples from the closed room were collected prior to arrival of the COVID-19 positive individuals, from distances between 4 and 12 feet from the individual, immediately after their departure and 2–6 h post their departure. The pre-arrival and post departure samples were collected from the place where the participants sat in the room. These experiments were conducted in September 2020. The details of air sampling conditions are mentioned in Table 2. Fig. 1 shows the schematic representing the sampling conditions in closed room.

2.3. Air sample collection from houses of home-quarantine COVID-19 patients

Air samples were collected from houses of four families with at least one member positive for COVID-19 at the time of sampling, during November–December 2020. Air samples were collected from various rooms, like bedroom, kitchen, living room, bathroom and lunch area, that were occupied by COVID-19 positive and negative members of the four families.

2.4. Swab sample collection

Nasopharyngeal swabs were collected in viral transfer medium (VTM) from the participants of the closed-room control study and the house study. The Ct values are shown in Table 2 and Data in brief Table 4. The swabs were processed according to standard procedures and SARS-CoV-2 viral RNA Ct values were determined.

2.5. Air sample collection

Air samples were collected on disposable gelatin filters (Sartorius, Cat. No. 17528-80-ACD) using AirPort MD8 air sampler (Sartorius, Cat. No. 16757). 1000 L of air was collected at a flow rate of 50 L per minute and a sampling time of 20 min. After sample collection, the gelatin membrane was aseptically removed, dissolved in 4 ml of Tris-EDTA buffer (10 mM Tris and 0.1 mM EDTA; pH

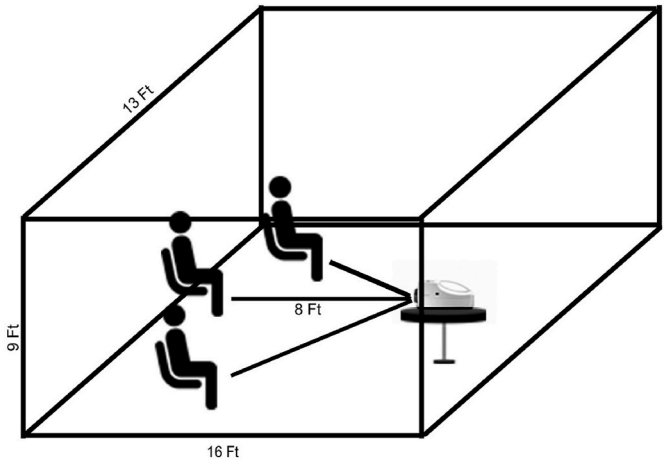

Fig. 1. Schematic representation of a room from where air samples were collected for analysis of SARS-CoV-2: COVID-19 positive individuals were instructed to sit in a closed room and air samples were collected from 4, 8 and 12 feet from them and analyzed for the presence of SARS-CoV-2 by PCR.

Table 1
Detection of SARS-CoV-2 at various locations of hospitals in Hyderabad and Mohali.

Hospital/Sampling No.	Air sampling locations	SARS-CoV-2 RNA detection
Hospital 1	COVID ICU 1	Yes
	COVID ICU 2	No
	Nurse Station	No
	COVID Ward	Yes
	Corridor	Yes
Hospital 2	Non-COVID Ward 1	No
	Non-COVID Ward 2	No
	COVID Nurse Station	No
	COVID ICU 1	No
	COVID ICU 2	No
	PPE Doffing Area	No
	COVID Room	No
	OP Corridor	No
Hospital 3: Sampling 1	Non-COVID Ward	No
	COVID Nurse Station	Yes
	Mortuary	Yes
	OP Corridor	No
	COVID Casualty	No
	PPE Doffing Area	No
	Non-COVID ICU	No
	COVID Room	No
	COVID ICU	No
Hospital 3: Sampling 2	OP Corridor	Yes
	Mortury	No
	COVID Casualty	No
	COVID ICU	Yes
	COVID Nurse Station	No
	PPE Doffing Area	No
	Non-COVID ICU	No
Hospital 3: Sampling 3	OP Corridor	No
	Mortuary	No
	Non-COVID ICU	No
	COVID ICU	Yes
	COVID Nurse Station	No
	PPE Doffing Area	No
Hospital 3: Sampling 4	COVID Room 1 Sample 1	No
	COVID Room 1 Sample 2	No
	COVID Room 1 Sample 3	Yes
	COVID Room 2 Sample 1	No
	COVID Room 2 Sample 2	Yes
	COVID Room 2 Sample 3	No
Hospital 3: Sampling 5	Non-COVID Ward	No
	COVID Casualty	No
	COVID ICU 1	Yes
	COVID ICU 2	No
	COVID Nurse Station 1	Yes
	COVID ICU 3	No
	ICU 4	Yes
	COVID Nurse Station 2	No
	COVID Room 1	Yes
	COVID Room 2	Yes
	COVID Room 3	Yes
	COVID Room 4	Yes
Hospital 3: Sampling 6	COVID Room 5	Yes
	COVID Room 6	Yes
	COVID Room 7	Yes
	COVID Room 8	No
Hospital 4: Sampling 1	Sample Collection Room	Yes
Hospital 5: Sampling 1	COVID Ward	Yes
	COVID ICU	Yes
Hospital 5: Sampling 2	Duty Dr's Room 1	Yes
	Duty Dr's Room 2	No
	COVID ICU Sample 1	Yes
	COVID ICU Sample 2	No
	COVID ICU Sample 3	No
	COVID ICU Sample 4	Yes
	COVID ICU Sample 5	Yes
	COVID ICU Sample 6	Yes

(*continued on next page*)

Table 1 (*continued*)

Hospital/Sampling No.	Air sampling locations	SARS-CoV-2 RNA detection
	General Ward Sample 1	No
	General Ward Sample 2	Yes
Hospital 6	General Ward	No
	COVID ICU Sample 1	No
	COVID ICU Sample 2	No
	COVID Ward	No
Hospital 4: Sampling 2	Sample Collection Area	No
	COVID Ward 1	No
	COVID Ward 2	Yes
Hospital 5: Sampling 3	COVID ICU	No
	COVID ICU Nurse Station	No
	COVID Ward	No

7.4) or VTM at 37 °C in a water bath for 10 min or till it completely dissolved. 1 ml of sample was used for RNA extraction and the remaining was stored at −80 °C. Samples collected during sampling 1 at Hospital 4 and 5 in Mohali, were collected using Whatman filter paper placed on 90 mm Petri dish using Merck's MAS-100® series air sampler, 1000 L at a rate of 100 L per minute.

2.6. RNA extraction and Real Time-RT-PCR

1 ml sample containing dissolved gelatin membrane was used for RNA extraction by guanidinium thiocyanate-phenol-chloroform extraction -column method. The sample was lysed using QIAzol Lysis Reagent (Qiagen, Cat. No. 79306) and incubated at room temperature for 10 min. After adding chloroform, mixing and centrifuging at 12,000×*g* for 10 min at 4 °C, the RNA containing aqueous layer was used for RNA isolation using QIAamp Viral RNA Mini kit (Qiagen; Cat No. ID: 52906) according to manufacturer's protocol. The extracted RNA was used for SARS-CoV-2 E gene, N gene and ORF1ab gene detection using Fosun COVID-19 RT-PCR Detection Kit (Shanghai Fosun Long March Medical Science Co., Ltd; Cat. No. PCSYHF03-a). RT-PCR was run on QuantStudio™ 5 Real Time PCR system. RT-PCR was done in triplicates for each sample and data was analyzed using Design and Analysis Software v1.5.1 QuantStudio™ 5. For sampling 1 of Hospital 4 and 5 at Mohali, RNA was isolated using AURA PURE Viral RNA Isolation Kit (Aura Biotechnologies Private Ltd., Cat. No. MNP-R004-100) and RT-PCR was performed using TRUPCR® COVID-19 Real-Time RT-PCR Kit (3B Blackbio Biotech India Ltd; Cat No. 3B304).

2.7. Infection of CCL-81 vero cells with SARS-CoV-2 collected from air

SARS-CoV-2 positive air samples were used for infecting CCL-81 Vero cells in Biosafety Level-3 (BSL-3) laboratory settings. The air samples in gelatinous medium (TE) that were stored at −80 °C, were first thawed at 37 °C and concentrated with 100 kDa centrifugal filter (37 °C) and resuspended in serum free DMEM. It must be noted that, since the samples had undergone a freeze-thaw cycle, some loss in culturable virus is expected. Before infection, the sample was filter-sterilized (0.45 μm) and added to Vero monolayers in 96-well plate. Three hours post-infection, the media was replaced with fresh 10% serum containing media. The infected cells were further incubated at 37 °C with 5% CO2 in a humidified chamber and cytopathic effects (CPE) were examined every 24 h for five to six days. Cells along with the supernatants were collected from those wells displaying CPE and transferred to fresh 48-well plate containing Vero monolayers for further propagation. This process of viral passaging and culture scale up continued for 5 passage and at each level viral supernatant collected and stored in −80 °C freezers for further use. For each passage viral supernatant was quantified by SARS-CoV-2 specific primers (LabGenomics; Labgun COVID-19 RT-PCR kit; CV9032B) against RdRP and E gene. The later passage supernatants with sufficient volume were titrated for infectious particle count by plaque-forming assay.

3. Results

3.1. Detection of SARS-CoV-2 RNA in hospital air samples

We analyzed a total of 80 air samples from various locations at hospitals from two cities in India- Hyderabad and Mohali. Air samples were collected from COVID ICUs, nurse stations, COVID wards, corridors, non-COVID wards, PPE doffing areas, COVID rooms, OP corridors, mortuary, COVID casualty areas, non-COVID ICUs and doctors' rooms from hospitals. Out of the 80 samples collected, 30 samples were positive for SARS-CoV-2 RNA (Table 1 and Data in brief Table 1). From Hyderabad, 57 air samples were collected from 3 hospitals. Out of 57, 30 samples showed the presence of SARS-CoV-2 RNA (Table 1 and Data in brief Table 1). From Mohali, 23 air samples were collected from 3 hospitals. Among these, 10 samples showed the presence of SARS-CoV-2 RNA (Table 1 and Data in brief Table 1).

A total of 57 air samples were collected from indicated locations of hospitals 1–3 in Hyderabad and analyzed for the presence of SARS-CoV-2 RNA. A total of 23 air samples were collected from indicated locations of hospitals 4–6 in Mohali and analyzed for the presence of SARS-CoV-2 RNA.

3.2. Detection of SARS-CoV-2 RNA in air in closed room experiments

To understand how far and for how long SARS-CoV-2 can be detected in the air, when COVID-19 positive individuals spend a short time in closed room, we analyzed air samples at different distances from COVID-19 positive individuals and at different time points. One or 3 participants were made to sit in one corner of a room with no perceived air flow for short span of time and air samples were collected at varying distances from them (Fig. 1). When one participant was present, he was positioned at a specific distance from the air sampler. When 3 participants were present in the room, they were positioned in such a manner that the distance between each participant and the air sampler was same. SARS-CoV-2 RNA was detected 5 out of 17 samples collected (Table 2). Importantly, samples collected post departure of the patients from the room were also positive for SARS-CoV-2 RNA.

7 COVID-19 positive individuals were asked to spend indicated time in a closed room during air sample collection. Each sampling was performed for 20 min. Air samples were collected at the indicated distances from the individuals and analyzed for the presence of SARS-CoV-2 RNA. N* indicates 'information not available'

3.3. Detection of SARS-CoV-2 RNA in air from rooms of houses of home-quarantined COVID-19 patients

To get a better insight about the airborne transmission of SARS-CoV-2, air samples were collected from the rooms of houses of home-quarantined COVID-19 patients. Samples were collected from houses of three families with one or more members infected with SARS-CoV-2 (Table 3). The first family consisted of 3 members. One of the members had symptoms like fever, sore throat, tiredness and cough, and was detected positive for COVID-19, one day prior to the study. The member was immediately room quarantined. Next day, nasopharyngeal swab samples were collected from all the three family members. The individual, who previously tested positive, was confirmed positive and the other two individuals in the family were found to be negative for COVID-19 (Data in brief Table 4) on the day of study. Air samples were collected from the bedroom occupied by the patient, other family member's bedroom, hall, kitchen and bathroom. Sample collected from the patient's bedroom, when he was not wearing mask and was not talking, was positive for SARS-CoV-2 RNA, whereas all other air samples were negative (Table 3).

The second family consisted of 5 members, of which 2 were reported COVID-19 positive one day prior to the study (Table 3). Both had fever previously, but were asymptomatic during the day of air sample collection. They were quarantined in separate rooms. Nasopharyngeal swab test on the day of air sampling confirmed the positivity of the two patients and the negativity of the remaining 3 members (Data in brief Table 4). Air samples were collected at a distance of 2 and 4 m from one of the positive individuals who wore mask and was conversing on phone during sample collection. Both the samples were negative for SARS-CoV-2. Air samples were collected at a distance of 1.5 and 3.5 m from second positive individual who was not wearing mask. Both the samples were found to be positive for SARS-CoV-2 RNA. Two more samples were collected from the house of this family-one from hall occupied by three COVID-19 negative individuals and the other from a room occupied by one COVID-19 negative individual. Both the samples were found negative for SARS-CoV-2 (Table 3).

The third family consisted of 6 members (Table 3). Prior to the study, only 2 members were known to be positive for SARS-CoV-2. The family members did not wear mask at home, were not room quarantined and interacted with each other verbally during the sampling event. On the first day of sampling, nasopharyngeal swabs were collected from all the six members and all of them were found to be positive for COVID-19 (Data in brief Table 4). Air samples were collected from 1.5 to 3 m from patient 1, who was previously known to be positive. Both the air samples were found to be positive for SARS-CoV-2 RNA. Another air sample, collected from the bedroom of patient 1, which was unoccupied by the patient during sample collection, was found to be positive for SARS-CoV-2 RNA. The room was vacated by the patient at least 2 h before sample collection, indicating that, SARS-CoV-2 can stay in the air of closed rooms for at least 2 h. Next day, another four air samples, collected from the hall and dining room occupied by the COVID-19 positive

Table 2
Analysis of air samples from closed room occupied by COVID-19 positive individuals.

No. of Individuals in room	Symptoms	Swab Ct value	Distance/Condition	SARS-CoV-2 detection in air	Time spent
1	Asymptomatic	28.87	4 feet	No	Sampling time
			12 feet	No	20 min + Sampling time
3	Asymptomatic	28.87, 31.76, 32.47	4 feet	Yes	Sampling time
			12 feet	No	20 min + Sampling time
			Pre-Arrival	No	0 min
			Departure	Yes	40 min
			Post-departure 2–6 h	Yes	40 min
1	Asymptomatic	27.57	4 feet	No	Sampling time
			8 feet	No	20 min + Sampling time
			12 feet	No	40 min + Sampling time
			Departure	No	60 min
3	Mild symptoms	29.1, 19.85, N*	4 feet	No	Sampling time
			8 feet	No	20 min + Sampling time
			12 feet	No	40 min + Sampling time
			Pre-Arrival	No	0 min
			Departure	Yes	60 min
			Post-departure 2–6 h	Yes	60 min

Table 3
Analysis of air samples collected from houses of COVID-19 positive individuals.

Family details	Air sampling condition	SARS-CoV-2 RNA detection
Family 1–3 Members, 1 Patient	Patient bedroom	Yes
	Family member's bedroom	No
	Bathroom	No
	Hall	No
	Kitchen	No
Family 2–5 Members, 2 Patients	4 m from Patient 1	No
	2 m from Patient 1	No
	3.5 m from Patient 2	Yes
	1.5 m from Patient 2	Yes
	Hall- Occupied by 3 COVID -ve individuals	No
	Room-Occupied by 1 COVID -ve individual	No
Family 3–6 members, 6 Patients	Sampling day 1–1.5 m from patient 1	Yes
	Sampling day 1–3 m from patient 1	Yes
	Sampling day 1- Patient 1 room, vacated 2 h before sampling	Yes
	Sampling day 2- Hall- Occupied by patients	Yes
	Sampling day 2- Dining room- Occupied by patients	Yes
	Sampling day 2- Bathroom- Unoccupied	Yes
	Sampling day 2- Bedroom- Unoccupied	Yes

individuals, and bathroom and bedroom, previously occupied, but unoccupied during sample collection, were found to be positive for SARS-CoV-2 RNA (Table 3).

Air samples were collected from different locations of houses of three families having at least one COVID-19 patient. Sampling time was 20 min.

Three SARS-CoV-2 RNA positive air samples from the third house study that had relatively lower Ct values (expected to have relatively higher viral load) were used for infecting vero cells in culture. Out of the three samples, one sample established culture. The details of the culture are provided in Data in brief Table 5.

4. Discussion

In the initial phases of the pandemic, the transmission of SARS-CoV-2 was largely thought to be through contact and droplet spread, in general public opinion. However, with studies reporting transmission among physically distanced individuals in closed spaces with air conditioning (Lu, Gu et al., 2020; Sommerstein, Fux et al., 2020) and the fact that, the viral spread could not be effectively curbed in spite of strict lockdowns in various countries of the world, raised the possibility of airborne transmission. This line of thought was substantiated by previous studies which detected viral RNA or live virus in the air (Bing, Zhang et al., 2018; Wu, Shen et al., 2010). Aerosol mediated transmission is not limited to human viruses, for example, in swine it is known that porcine respiratory and reproductive syndrome virus (PRRSV) is transmitted via an aerosol route (Nirmala, Alves et al., 2021). CDC released a statement acknowledging the possibility of air borne transmission in certain scenarios for SARS-CoV-2 (https://www.cdc.gov/coronavirus/2019-ncov/more/scientific-brief-sars-cov-2.html).

In our study, SARS-CoV-2 could be detected in the air collected from various COVID care areas with no specific predilection towards ICU/non-ICU areas in the hospitals ($P = 0.5492$; Table 4). The virus could not be detected in any of the non-COVID care areas. The positivity rate was found to be more when the number of COVID patients were higher in the room. The SARS-CoV-2 RNA positivity percentage was 58% when 2 or more COVID-19 patients were present in a room, in contrast to 24.62% when 1 or 0 COVID-19 patients occupied the room. ($P = 0.0185$; Table 5). A point to be highlighted from the hospital experiments was that in many of SARS-CoV-2 RNA positive samples, the air sampler was at least 8 feet away from the nearest patient. As there is no record on events that occurred in the sampling area before the sample collection began, we are unable to infer this finding. But this may be an indicator that long term presence of COVID positive patients in an enclosed space may contribute to a significant increase in aerosol burden in the air. These findings have significant implications in the current situation, when many countries have relaxed the restrictions on public mobility and interactions. In many densely populated nations where the recommended physical distancing norms may be difficult to implement in public/office spaces, distancing as much as possible with usage of masks should be actively promoted.

Table 4
Table showing the data for total number of SARS-CoV-2 positive samples in COVID-ICU and non-ICU areas in hospitals in Hyderabad and Mohali.

Criteria	Hyderabad	Mohali	Total	% positive
Total no. of air samples collected	57	23	80	37.5
Total no. of SARS-CoV-2 RNA positive air samples	20	10	30	
Total no. of air samples collected from COVID ICUs	11	11	22	45.45
Total no. of SARS-CoV-2 RNA positive air samples from COVID ICUs	5	5	10	
Total no. of air samples collected from non- ICUs	46	12	58	34.48
Total no. of SARS-CoV-2 RNA positive air samples from non-ICUs	15	5	20	

Table 5
Table showing percentage positivity for air samples with SARS-CoV-2 RNA when 2 or more COVID patients occupied the room versus 1 or 0 COVID patients during sample collection.

Criteria	Hyderabad hospitals	Mohali hospitals	Closed Room	House	Total	% positivity for total	Closed room + house	% positivity for closed room + house
Total no. of air samples collected	57	23	17	18	115	39.13	35	42.86
Total no. of SARS-CoV-2 RNA positive air samples	20	10	5	10	45		15	
Total no. of air samples collected from rooms occupied by 2 or more COVID patients	19	15	9	7	50	58	16	75
Total no. of SARS-CoV-2 RNA positive air samples from rooms occupied by 2 or more COVID patients	10	7	5	7	29		12	
Total no. of air samples collected from rooms occupied by 1 or 0 COVID patients	38	8	8	11	65	24.62	19	15.79
Total no. of SARS-CoV-2 RNA positive air samples from rooms occupied by 1 or 0 COVID patients	10	3	0	3	16		3	

Significance was calculated between the number of SARS-CoV-2 RNA positive samples in COVID-ICU and non-ICU groups, using Odd's ratio with 95% confidence interval. P = 0.5492.

Significance was calculated 1) between the number of SARS-CoV-2 RNA positive samples from the rooms (including hospitals) with 2 or more patients versus room (including hospitals) with 1 or 0 COVID-19 patients, using Odd's ratio with 95% confidence interval; P = 0.0185.2) between the number of SARS-CoV-2 RNA positive samples from the rooms (only closed rooms + houses) with 2 or more patients versus room (only closed rooms + houses) with 1 or 0 COVID-19 patients, using Odd's ratio with 95% confidence interval; P = 0.0326.

The novelty of this study lies in the closed-room control experiments. Most of the previous studies have not executed this kind of control experiment where the COVID-19 patients spent short time in a closed room and air samples are analyzed for SARS-CoV-2 RNA. Since the only source of SARS-CoV-2 RNA in the air in the closed room are the patients who are present in the room during air sample collection, the PCR signal in the air samples in this condition is from the viral particles released by the patients and not from RNA contamination from any other source. The detection of SARS-CoV-2 RNA in the air in the closed-control-rooms and the rooms of houses of home quarantined COVID-19 patients, suggest that SARS-CoV-2 is released by COVID-19 patients in the air and that it can stay in the air for some time in absence of ventilation in the closed spaces. It also indicates that the chances of finding SARS-CoV-2 in the air in closed room are higher if more number of COVID-19 positive individuals occupy the room. The SARS-CoV-2 RNA positivity percentage was 75% when 2 or more COVID-19 patients were present in a room in closed-room control study and the house study, in contrast to 15.79% when 1 or 0 COVID-19 patients occupied the room in these studies. (P = 0.0326; Table 5). Our observations are concurrent with previous studies that suggest that the concentration of SARS-CoV-2 RNA is higher in indoor air as compared to outdoor air; and in indoor, it is higher in hospital and healthcare settings that host more number of COVID-19 patients, as compared to that in community indoor settings (Dinoi, Feltracco et al., 2022).

It is to be noted that many of the air samples from hospitals and closed room experiments showed PCR signal for one of the SARS-CoV-2 genes or had very high Ct values. The possibility that live infectious SARS-CoV-2 being actually present in these air samples cannot be ruled out completely considering the diluting effect due to diffusion of the virus in the air and the fact that only 1000 L (1 cubic meter) of air was sampled each time. The limit of detection (LoD) for SARS-CoV-2 RNA in air is 1200 copies in our experimental setup. In this study, out of the 3 air samples with relatively lower Ct values, we could establish live culture for one of the air samples. Another study by Nannu Shankar et al. also attempted culturing of SARS-CoV-2 using vero cells, but their cells were contaminated by Human adenovirus B3 (HAdVB3) that killed the Vero E6 cell cultures (Nannu Shankar, Witanachchi et al., 2022). This is because the samples collected from air are mixed samples consisting of other bacteria and virus along with SARS-CoV-2. Whether initiating an infection in an individual has a different viral titer threshold from that needed for establishing a virus culture, is still unclear. A study found that an intranasal dose of 100 PFU of SARS-CoV-2 was sufficient to infect Syrian hamsters (Brocato, Principe et al., 2020). Another computational study estimated that SARS-CoV-2 particles in the range of hundreds is sufficient to establish infection in humans (Basu, 2021). Even though virus is diluted in the air, considering that such a low number of viral particles is sufficient to cause infection, aerosol mediated transmission can contribute substantially to the infection load due to SARS-CoV2.

5. Conclusions

Our results indicate that SARS-CoV-2 is released by COVID-19 patients in the air. If higher number of symptomatic COVID-19 patients are present in a closed space, the chances of finding SARS-CoV-2 in air are higher. SARS-CoV-2 released by COVID-19 patients in air can be infective. Following physical distancing along with wearing masks can help in preventing the spread of infection.

Author contributions

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Ethical approval

The study was approved by the Institutional Ethics Committee of CSIR- Centre for Cellular and Molecular Biology (IEC-83/2020). Informed consent from subjects was taken wherever required.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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